DNA Barcoding of Sea Slugs (*Nudibranch*)

Anna Vu

MENTORED BY

Dr. Chelsey Mckenna, College of Southern Nevada Biological Science Department

OVERVIEW

This research focuses on extracting, amplifying, and blasting the CO1 gene of sea slugs (*Nudibranch*) for DNA barcoding.

INTRODUCTION

DNA barcoding provides a quick, reliable, and basic approach to retrieving the identity of numerous species stored in accessible online databases. The goal of this research is to identify four *Nudibranchs* through DNA barcoding. As DNA barcoding focuses on a standardized CO1 gene for most species, database results will demonstrate if this species has been entered into a database. The level of dissimilarity between sequences of similar species stimulates the possibility that this research could add to the bioinformatic evidence of new *Nudibranch* species to the various DNA barcoding archives. As *Nudibranchs* number over 2000 species, the identification of more species will aid in the growth of current information on *Nudibranchs*.

WETLAB METHODS

-**PCR (2 rounds)**: Utilized extraction reverse &forward primers, DNA of 8 different *Nudibranchs* around 2mm, PCR beads (Taq polymerase), and a PCR machine with temperature settings of 95°C (denature), 45°C (anneal), and 72° C (adds nucleotides) for 30 cycles to produce a billion copies of the CO1 gene.

-**Gel Electrophoresis (2 rounds)**: Utilized 1% agarose gel in TAE buffer with wells. A successful process will generate DNA fragments shown with fluorescent bands. Samples demonstrating bands were sent to GeneWiz, a company that sequences DNA (from PCR) to nucleotides.

BIOINFORMATICS METHODS/ FIGURES

Utilized DNA Subway to trim DNA sequences, trim a DNA consensus (removed most mismatches between forward and reverse gene), BLAST (Basic Local Alignment Search Tool: generates nucleotide mismatches), and analyze sequences between similar *Nudibranchs* and unrelated marine invertebrates.

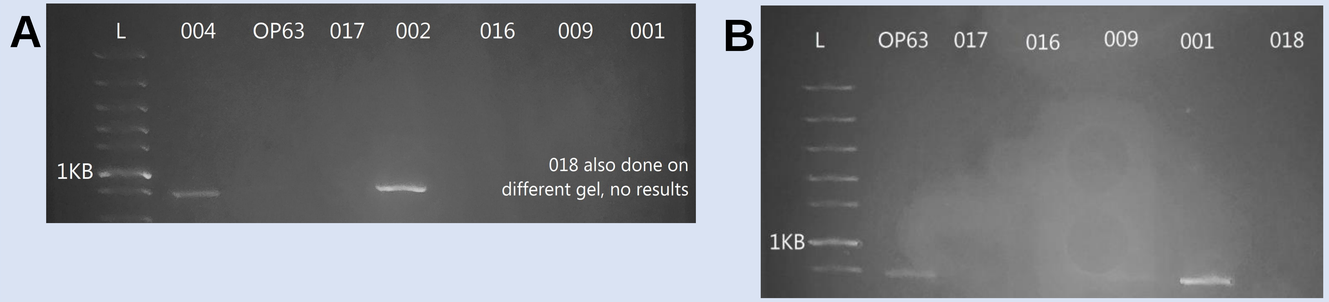
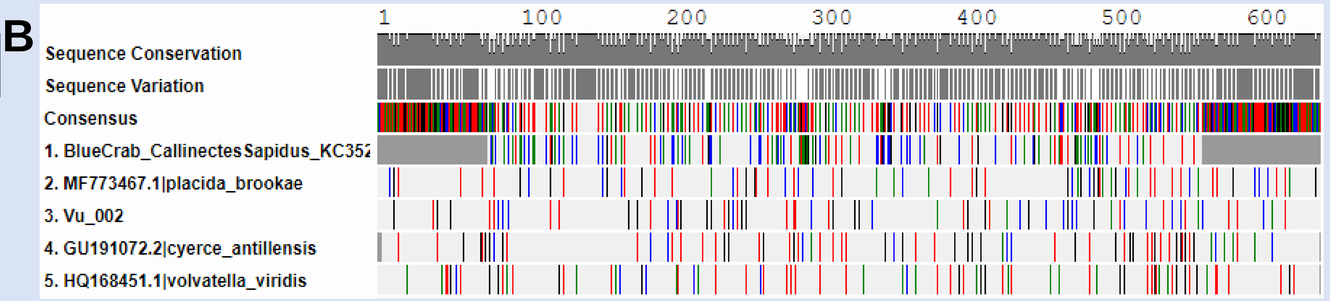


Figure 1: (A) Round 1 of gel electrophoresis, two *Nudibranchs'* DNA post-PCR were successful
(B) Round 2 of gel electrophoresis, two *Nudibranchs'* DNA post-PCR were successful

A

Details	Aln. Length	Bit Score	e	Mis-matches
Placida brookae isolate JCP02 cytochrome c oxidase subunit 1 (CO1) gene, partial cds - Placida brookae isolate JCP02 cytochrome c oxidase subunit 1 (CO1) gene, partial cds	651	715	0.0	102

B



C

	C	1	2	3	4	5
C	-	75.66	89.55	90.17	89.64	89.08
1	75.66	-	72.21	68.56	68.36	69.57
2	89.55	72.21	-	84.33	82.69	81.69
3	90.17	68.56	84.33	-	83.46	83.08
4	89.64	68.36	82.69	83.46	-	83.77
5	89.08	69.57	81.69	83.08	83.77	-

Figure 2:
(A) Sample 002's blast results—102 nucleotide mismatches
(B) MUSCLE (MUltiple Sequence Comparison by Log- Expectation) results—comparison of sample 002 with similar species and a marine invertebrate species, Blue Crab (*Callinectes Sapidus*).
(C) Sequence similarity percentage between sample 002, *Placida brookae*, *Callinectes Sapidus*, *Cyerce antillensis*, and *Volvatella viridis*

RESULTS

Two rounds of PCR yielded four (out of eight) viable *Nudibranchs* for sequencing **(Figure 1A, 1B).** Through this BLAST, we identified the closest species to the sequences extracted from the four *Nudibranchs.* For example, in **Figures 2B & 2C**, sample 002's sequences are the most similar to *Cyerce antillensis* with an 83.46% similarity. The additional comparison of *Nudibranchs* to marine invertebrates highlights the differences between the two species and solidifies the four *Nudibranchs* obtained as part of the sea slug genus.

CONCLUSION

Most of the four *Nudibranchs* (except sample OP63) displayed high levels of nucleotide mismatches, particularly with sample 002 **(Figure 2A).** This suggests evidence of a new species of sea slugs. The *Nudibranchs* will be sent to be identified by researchers specializing in the morphological/ phenotypic traits of *Nudibranchs*. If results relay the discovery of a new unidentified species, the trimmed DNA sequences from this research will be placed into the barcoding databases (i.e. GenBank).

FUTURE DIRECTIONS

-Obtain sea slugs (*Nudibranch)* with unknown backgrounds to extract, amplify, and blast to compare DNA sequences among online barcoding databases.

-Obtain samples of fungi from unknown backgrounds to extract, amplify, and blast to compare DNA sequences among the online barcoding databases.

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Aeronautics and Space Administration under Grant No. 80NSSC20M00043.